SPIEGLER'S "WHITE MELANIN" AS RELATED TO DOMINANT OR RECESSIVE WHITE[1]

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INTRODUCTION

THE study of melanin has interested a great number of chemists during the last century and of especial interest was the announcement by Spiegler,[2] in 1904, that he had succeeded in obtaining a "white melanin" from sheep's wool and white horse hair.

The question of white plumage and hair color has been widely studied from breeding standpoints—attracting unusual interest from the fact that there are undoubtedly two varieties of white, one of which is dominant and the other recessive. The reason for this peculiarity seemed, therefore, to be explained by the discovery of the "white melanin." In the light of this new knowledge it would seem that one variety of white was produced by the presence of a white coloring matter and that this would be dominant in a cross with another color having a weaker determiner. The recessive white, however, would be recessive because there would be an entire absence of pigment and would therefore be a case of dominance of color over absence of color.

Riddle,[3] in referring to Spiegler's work, seems to take this view, as does also Spillman.[4] Spiegler,[5] in a later paper, while giving no further experimental work on

[1] Used in the Mendelian sense. [Contribution from the Biochemical Laboratory of the Station for Experimental Evolution, The Carnegie Institution of Washington.]

[2] Spiegler, Hofmeister's *Beitr. z. Chem. Physiol. u. Path.*, 4, 40, 1904.

[3] Riddle, *Biol. Bull.*, 16, 328, 1909.

[4] Spillman, this journal, 44, 119, 1910.

[5] Spiegler, Hofmeister's *Beitr. z. Chem. Physiol. u. Path.*, 10, 253, 1907.

"white melanin" seems also to suggest that the two whites are due to the presence and absence of melanin. He states:

It is readily understandable that white horse hair can not be without pigment. We know pigmentless hair, *i. e.*, albinos, these have apparently the natural color of the keratin (Hornrohstoffs) from which the hair is formed, modified by a special morphological condition. These questions need a more searching study. We must now determine whether albino hair gives the same oxidation products as pigmented hair. It is in this manner that we can finally decide whether the color of gray hair is due, as we have previously supposed, to disappearance of pigment and air content or rather to the change of the darker pigments into lighter ones.

It is in this condition that the study was left. Inasmuch as this question is of the utmost importance from the standpoint of heredity, the work of melanin investigation has been taken up in this laboratory.

In a study of Spiegler's[6] paper the most noteworthy detail which appears to be wanting is a comparison between the percentage of black pigment in the black wool or hair and the percentage of "white melanin" found in the white varieties. Spiegler gives the method of isolation as practically the same for both varieties, but does not mention the yields of melanin obtained. He, then, gives his analytical data leading to an empiric formula of $C_{50}H_{58}N_8SO_{12}$ calculated to ash-free (ash = 9.8 per cent.) melanin from black horse hair and $C_{45}H_{78}N_{10}SO_{20}$ calculated to ash-free (ash = 16.28 per cent.) "white melanin" from white horse hair.[7] He further states:

The black pigment body with the simplest formula of $C_{50}H_{58}N_8SO_{12}$ and the light pigment body of $C_{45}H_{78}N_{10}SO_{20}$ differ, as the analytical data show *only a little* ("ein Geringes") *and it is very apparent that they are identical in nucleus* ("im Kerne"), and that the different color is due to the entrance of a chromatic group. Very apparent is the great difference in hydrogen content. The whlte pigment contains much more hydrogen, oxygen and even nitrogen, while the carbon-poor one is the black. The light pigment body is at the same time the oxidation and reduction product of the darker one.

[6] *Loc. cit.*

[7] His formula for melanin from black and white wool are respectively $C_{46}H_{68}N_8SO_{20}$ (ash = 10.85 per cent.) and $C_{61}H_{98}N_{10}SO_{20}$ (ash = 2.30 per cent.).

In other places, however, he prefers to call the white body an *oxidized black pigment*. In his paper no comparison is made of the black and white wool products; here we have formulæ assigned by Spiegler as $C_{46}H_{68}N_8SO_{20}$ and $C_{61}H_{98}N_{10}SO_{20}$, respectively. In this case white *could not be an oxidized black*, neither does the lower carbon percentage belong to the black. It is incomprehensible to the author why Spiegler should assert that "*it is apparent that both are identical in nucleus.*" The only point of identity which is *apparent* is that the same elements enter into the composition of each, but the proportions are so widely different that no close relationship seems possible. Coupling this with the facts that from black wool, treated in a manner very closely resembling Spiegler's method, the author has obtained 1.84 per cent. of black melanin, while from white wool only 0.06 per cent. of a grayish-brown[8] body was obtained by an exactly similar method; and also that albino hair (from white rabbits), obtained through the courtesy of Dr. Castle of Harvard College, gave 0.03 per cent. of a grayish-brown body; feathers from a recessive (albino) fowl (silky) gave 0.155 per cent. of a similar body and feathers from a dominant white fowl (white Leghorn) gave 0.195 per cent., it appears that Spiegler's "white melanin" is not a substance belonging to the melanin class, but is a product produced from the keratin by the action of alkalies. The author has been able to find no data as to the actual percentage of keratin in the hair or feathers of the various animals, but it seems probable that the coarser the covering of the animal, the greater the percentage of keratin. Thus in the fowls we find the coarse ribs of the feathers, which are composed almost wholly of keratin, while in the white rabbit the hair is very fine and silky and contains, supposedly, less keratin than the intermediate wool of sheep, which is more similar to the rabbit hair. The same holds true of the decomposition product found if we assume it is due

[8] Spiegler describes his "pigment" as "a light gray brown powder."

to the keratin—the coarser the structure of the coat the larger was the percentage of the decomposition product. Even if this view is not correct we know that the various keratins do not have the same composition,[9] and, therefore, we should look for a variation in any one decomposition product.

METHOD OF ISOLATION

A weight of wool was boiled with a 10-per-cent. solution of sodium hydrate[10] in the proportion of 300 grams to 1 liter for four hours. The solution was then poured into water, strongly acidified with hydrochloric acid[11] and the precipitate allowed to settle. The supernatant liquid was syphoned off and the precipitate washed by decantation. The precipitate was then stirred with from 5 to 10 liters of 0.2-per-cent. sodium hydrate solution and filtered. The filtrate was precipitated by hydrochloric acid and allowed to settle, the liquid syphoned off and the precipitate dissolved in one liter 0.2-per-cent. sodium hydrate solution and again filtered, precipitated with hydrochloric acid, washed free of chlorides, dried at 100° and, lastly, extracted with carbon disulphide, alcohol and ether in Soxhlet apparatus, then dried at 105° and weighed.

DISCUSSION

From the table given below it can be seen that "white melanin" does not exist in either recessive or dominant whites, but that there is some product *formed by the decomposition of the keratin,* which behaves like a melanin, *i. e.,* is soluble in alkali and insoluble in acids or neutral solvents; perhaps this may be shown to belong to the melanin class, but it is at least common to all white plumage and hair.

[9] See Hoppe-Seiler's "Handbuch der Physiologisch und Pathologisch Chemischen Analyse," Berlin, 1909, pp. 518–519.

[10] An exhaustive research as to the effect of various strengths of sodium hydrate solution upon melanin is in progress in this laboratory, the details of which will soon be ready for publication.

[11] A copious evolution of hydrogen sulphide was observed in each case

The results obtained are given in the following table.

Substance.	Weight.	Vol. alkali.	Strength alkali.	"Pigment" found.	Per cent. found.
1. Black wool[12]	400 gr.	1340 c.c.	10%	7.35 gr.	1.84
2. White wool[13]	500	1675	10	0.30	0.06
3. " " [14]	500	1675	10	0.30	0.06
4. " rabbit hair[12]	100	350	10	0.03	0.03
5. Silky feathers[12]	110	350	10	0.17	0.155
6. " "	105	700	5	0.24	0.22
7. " "	95	3000	1	0.16	0.168
8. White leghorn feathers[13]	46	200	10	0.09	0.195
9. Cow's horn (colorless)	40	200	10	Lost before weighing, but present in appreciable amount.	

If the theory of v. Furth[15] is correct that melanin formation is the product of an oxydase acting upon an oxidizable chromogen, it would appear very probable that dominant whiteness is due to the presence of an inhibitory enzyme[16] in the epithelial cells which prevents the action of the oxydase, and that recessive whites differ by having neither the power to produce pigments, *i. e.*, lack of oxydase or chromogen, or both, nor do they possess the anti-oxydase which distinguishes the dominant whites. This being the case, the one type would be always dominant, *its determiner being the anti-enzyme*, and the other type (*i. e.*, albinos) would of necessity be recessive, inasmuch as, while they lack the power to produce pigment, they also are without means of inhibiting pigment production when the elements for its

[12] Recessive in the Mendelian sense.

[13] Dominant in the Mendelian sense.

[14] Had been previously digested at 40° for 48 hours with 9 liters of 0.2-per-cent. hydrochloric acid containing 18 grams of pepsin (Merck's "scales" 1 to 3,000).

[15] v. Furth u. Schneider Hofmeister's *Beitr. z. Chem. Physiol. u. Path.*, 1, 229, 1902. v. Furth u. Jerusalem, *ibid.*, 10, 131, 1907.

[16] For the literature of the anti-enzymes see Vernon, "Intracellular Enzymes," London, 1908, pp. 208–211, and Kastle, "The Oxidases," Bull. No. 59, Hyg. Lab., U. S. Pub. Health and Mar.-Hosp. Serv., Wash., pp. 66 and 87, 1910.

formation are present. Davenport[17] has already put forward a view very similar to the above and it is hoped that in the near future this laboratory will have sufficient data to test this hypothesis.

SUMMARY

1. Dominant and recessive whites in the Mendelian sense, have no relation to the presence of Speigler's "white melanin."

2. The "white melanin" was found to be present in all forms of keratin structure which were studied, but in very small amounts as compared with true melanin from black wool.

3. In view of the small percentage of "white melanin" found, Spiegler's view that it is an "oxidized black" seems impossible; neither is this view upheld by a study of Spiegler's work.

4. It seems highly probable that Spiegler's "white melanin" bears no relation to true melanins, but is a decomposition product of the keratin.

5. A theory is advanced that dominant whites are due to the presence of an anti-oxydase which prevents pigment formation; recessive whites, on the other hand, have neither power to form pigments nor to inhibit the formation.

[17] Davenport, Report Am. Breeders' Assoc., 5, 382, 1909.